

Carbol Fuchsin Stain, Kinyoun for AFB - Technical Memo

SOLUTION:	250 ml	500 ml
Carbol Fuchsin Stain, Kinyoun	Part 1031A	Part 1031B

Additionally Needed:

Acid Fast Bacteria (AFB) Control Slides	Part 4011
Acid Alcohol 1%	Part 10011
Methylene Blue Stain 0.14%, Alcoholic	Part 12401
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Carbol Fuchsin Stain, Kinyoun is used in the Kinyoun AFB Stain to demonstrate the presence of acid-fast mycobacteria in tissue sections. Carbol Fuchsin Stain, Kinyoun is a concentrated phenol and basic fuchsin solution that works to permeate the lipid capsule of acid-fast organisms, rendering them resistant to acid alcohol decolorization.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Carbol Fuchsin Stain, Kinyoun with filter paper before use.

STAINING PROCEDURE:

3. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #1 and #2.
4. Stain in freshly filtered Carbol Fuchsin Stain, Kinyoun for 15 minutes at room temperature. Keep solution covered.
 - a. See Procedure Note #3.
5. Wash in running tap water for 2 to 3 minutes.
6. Differentiate in Acid Alcohol 1% (10011) until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
7. Wash in running tap water 3 to 5 minutes; rinse in distilled water.
8. Counterstain in Methylene Blue Stain 0.14%, Alcoholic (12401); 3-6 dips.
 - a. See Procedure Note #4.
9. Wash in running tap water for 1 minute; rinse in distilled water.
10. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Acid-fast bacteria	Bright red
Background	Pale blue

PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. Sections can remain in Carbol Fuchsin Stain, Kinyoun for up to 60 minutes without adverse effect. Additional differentiation may be required in Step #6.
4. If preferred, Light Green SF Yellowish Stain 0.1%, Aqueous (12203) can be used as a counterstain in place of Methylene Blue.
 - a. Stain for 3-6 dips.
 - b. Rinse with one quick dip in distilled water or proceed directly to Step #10 without a distilled water rinse.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 224-226.
2. Kinyoun. J.J. "A Note on Uhlenhuths Method for Sputum Examination, for Tubercle Bacilli." *American Journal of Public Health* 5.9 (1915). 867-870.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 236-237.
4. Modifications developed by Newcomer Supply Laboratory.